Reference: *Biol. Bull.* **162:** 311–332. (June, 1982)

A SURVEY OF THE RESPONSES OF BIVALVE HEARTS TO THE MOLLUSCAN NEUROPEPTIDE FMRFAMIDE AND TO 5-HYDROXYTRYPTAMINE

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Abstract

Ventricles from 50 species of bivalved molluscs were surveyed for their mechanical responses to the molluscan neuropeptide FMRFamide (Phe-Met-Arg-Phe-NH_2) and to 5-hydroxytryptamine (5HT). Both were predominantly cardioexcitatory, but neither was exclusively so. FMRFamide was inhibitory or weakly excitatory more often than 5HT, and such effects were most common in the subclasses Paleoheterodonta and Heterodonta. In contrast, 5HT was only rarely inhibitory or even weakly excitatory, and such effects were most common in the subclass Pteriomorphia. The responses to FMRFamide or 5HT were strikingly uniform in some bivalve families, but characteristically diverse in others. Thus, FMRFamide is neither a general cardioexcitor nor a general serotonomimetic agent.

Introduction

The molluscan neuropeptide FMRFamide (Phe-Met-Arg-Phe-NH_2), isolated from the clam *Macrocallista nimbosa*, increases the force and frequency of beat of isolated *Macrocallista* or *Mercenaria mercenaria* ventricles. These actions are identical to those of 5-hydroxytryptamine (5HT; serotonin) (Price and Greenberg, 1977). Both FMRFamide and 5HT stimulate adenylate cyclase activity and elevate cyclic adenosine monophosphate (cAMP) levels in these hearts (Higgins, 1977; Higgins *et al.*, 1978), even though the two agonists act at pharmacologically distinguishable receptor sites (Price and Greenberg, 1977). These observations suggested that FMRFamide might function as a long distance, long duration serotonomimetic agent (Price and Greenberg, 1977).

To test this hypothesis, we began to survey the effects of FMRFamide and 5HT on the mechanical activity of hearts from many species of bivalved molluscs. It soon became clear that FMRFamide is neither an unswerving mimic of 5HT nor an inevitable cardioexcitor. These general conclusions and representative data have been presented to the American Society of Zoologists (Painter *et al.*, 1979) and reviewed (Greenberg and Price, 1979, 1980; Greenberg *et al.*, 1982), but the survey itself, now completed, has never been published. These primary data are presented here.

Received 20 February 1981; accepted 12 March 1982.

Abbreviations: FMRFamide, Phe-Met-Arg-Phe-NH_2 (from the one letter abbreviations for amino acids approved by the IUPAC-IUB Commission on Biochemical Nomenclature, and after Price and Greenberg, 1977); 5HT, 5-hydroxytryptamine; DRP, dose-response profile; SRI, species response index.

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Materials and Methods

Animals

Most freshwater bivalves were obtained from the Ochlockonee River and Lake Talquin in Leon County, Florida; but *Ligumia recta* and *Lampsilis ovata ventricosa* were collected from the Wisconsin River in Richland County, Wisconsin, and *Ligumia subrostrata* from a pond in Livingston Parish, Louisiana. Gulf coast marine and brackish water animals were taken from the estuaries, marshes, and sand bars of north Florida. Bivalves of the northeastern Atlantic coast were purchased from Northeast Marine Specimens Co. of Bourne, Massachusetts; Pacific coast animals from Pacific Bio-Marine Co. of Venice, California; and *Lima scabra* from Gulf Specimens Co. of Panacea, Florida. Most Panamanian species were purchased at a fish market in Panama City, Panama; *Ostrea palmula* were collected from Miraflores Third Locks Lake.

All animals were maintained in aerated aquaria for several days before use. The marine species were kept in seawater, the brackish water bivalves in diluted seawater (375 mOsm for *Ostrea palmula*, 300 mOsm for both *Rangia cuneata* and *Polymesoda caroliniana*), and the freshwater animals in river water. The osmotic concentrations of the media were monitored with a freezing point depression osmometer (Precision Instruments, Osmette) and were replaced as needed. The Atlantic and Pacific coast animals were maintained and tested at 16°C, but all others at 21°C.

Procedure

The ventricles were prepared according to the procedures of Welsh and Taub (1948) and Greenberg (1965). One end of each heart was secured to a hook at the bottom of an organ bath, and the other end was attached via a spring to a force-displacement transducer (Grass Model FT-03). Mechanical activity was recorded on a Grass Model 79C polygraph.

The hearts of marine species were superfused with natural seawater, and the others with an appropriately diluted seawater: 50 mOsm for the freshwater species (Deaton and Greenberg, 1980), 300 mOsm for *R. cuneata* and *P. caroliniana*, and 375 mOsm for *O. palmula*. Aeration and mixing were provided by a magnetic stirring bar at the bottom of the bath.

Each heart was treated with a sequence of increasing doses of FMRFamide and 5HT. The drugs were added directly to the bathing medium, and the medium was changed between successive doses. The full range of FMRFamide concentrations was usually tested before 5HT; but we reversed the order of application in a number of preliminary experiments and found no effect on the responses to either agent. All doses are expressed as final molar concentrations in the bathing medium.

Chemicals

The drugs used in this study include: FMRFamide (Peninsula Labs), and 5-hydroxytryptamine creatinine sulfate (5HT) (Sigma).

Results

This survey includes over 9000 responses which vary qualitatively with species, drug, and dose tested. The major problem has been to characterize and organize

these disparate responses so that pharmacological and taxonomic comparisons could be made. We have approached the problem as follows.

Individual responses

To reduce the qualitative variation between individual responses, drug effects have been resolved into "excitatory" and "inhibitory" components, as detailed below.

Increases in frequency or diastolic tone were classified as excitations, while decreases were inhibitions. Frequency and diastolic tone usually changed in the same direction, although tone was affected mainly at higher doses.

Changes in amplitude were more difficult to assess since contractile force is often inversely related to frequency (molluscs: Greenberg, 1963; mammals: Blinks and Koch-Weser, 1961). Therefore, changes in amplitude were used to classify responses only when neither frequency nor diastolic tone were affected. In such cases, positive inotropy was excitation and negative inotropy inhibition.

An induced arrhythmia, although rare, was classified as an inhibition. An improvement in rhythmicity was more common, especially at threshold doses, and was designated an excitation.

Each response was thus classified as an excitation, an inhibition, or a mixed (*i.e.,* containing both excitatory and inhibitory components) response. Figure 1 clearly shows that FMRFamide and 5HT produced qualitatively similar excitatory, inhibitory, and mixed responses, though not necessarily in the same species or at the same concentration.

Dose-response profiles (DRPs)

A DRP is the set of responses of any preparation to a specified sequence of doses of agonist (1×10^{-10} to 1×10^{-6} *M*). Since most hearts were not exposed to doses exceeding 1×10^{-6} *M*, the effects of those high doses are not included in the profiles.

Some DRPs consisted entirely of qualitatively similar responses, either excitatory or inhibitory. Such profiles can be represented by a conventional dose-response curve and characterized by an ED_{50}. The individual responses of many DRPs changed qualitatively with dose, however, and these profiles are not amenable to the usual graphical representations. We had to develop new techniques to deal with these kinds of profiles.

Examples of FMRFamide and 5HT DRPs are illustrated in Figures 2 and 3, respectively. Each DRP was categorized as excitatory, inhibitory, or complex (*i.e.,* having both excitatory and inhibitory components, and changing qualitatively with dose) based on the sequence of effects observed.

Species responses

The species response is the set of DRPs to FMRFamide or 5HT obtained from a single species. If the species response is uniform, the same sequence of effects was observed in each preparation; *i.e.,* the DRPs are similar. If the species response is diverse, the DRPs differed qualitatively among preparations. In either case, a preponderant effect (excitation or inhibition) is usually identifiable; when it is not, the species response is complex.

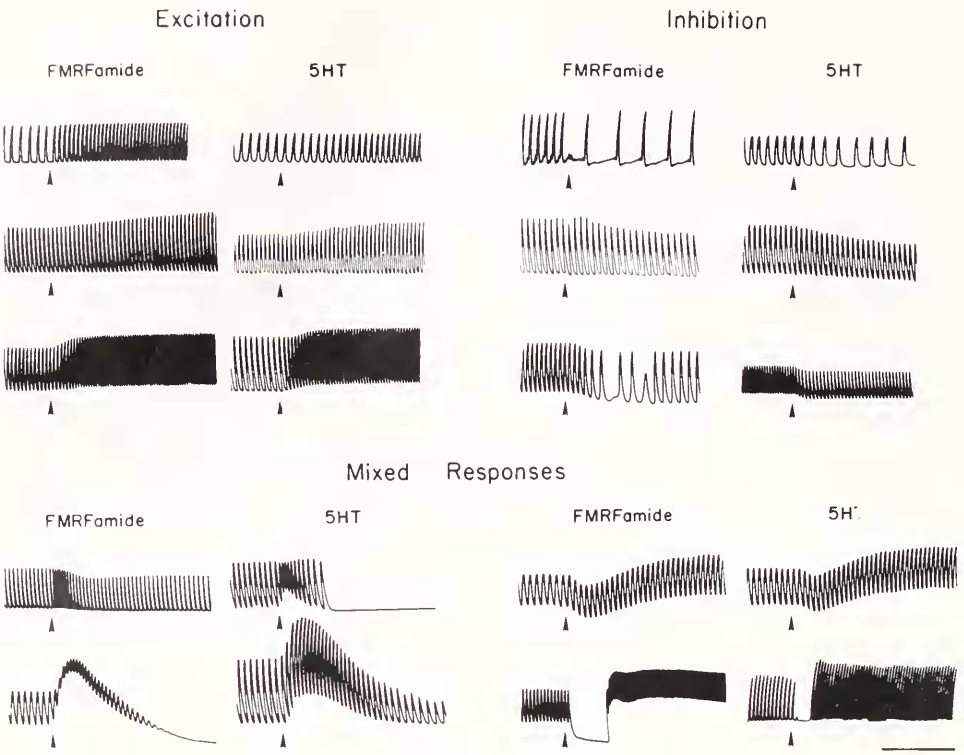


FIGURE 1. Examples of the responses of bivalve hearts to FMRFamide and 5HT. The species are identified from top to bottom and left to right. FMRFamide excitation: *Geukensia demissa granosissima*, 1×10^{-7} *M*; *Dinocardium robustum*, 3×10^{-7} *M*; *Modiolus squamosus*, 1×10^{-7} *M*. 5HT excitation: *Mytella guyanensis*, 3×10^{-7} *M*; *Lampsilis ovata ventricosa*, 1×10^{-6} *M*; *Mytilus edulis*, 1×10^{-6} *M*. FMRFamide inhibition: *Elliptio icterina*, 1×10^{-7} *M*; *Lampsilis ovata ventricosa*, 1×10^{-8} *M*; *Cyrtopleura costata*, 1×10^{-7} *M*. 5HT inhibition: *Geukensia demissa granosissima*, 1×10^{-8} *M*; *Lampsilis ovata ventricosa*, 1×10^{-5} *M*; *Rangia cuneata*, 1×10^{-6} *M*. FMRFamide mixed responses: *Rangia cuneata*, 1×10^{-7} *M*; *Pseudochama exogyra*, 1×10^{-6} *M*; *Corbicula manilensis*, 3×10^{-10} *M*; *Noetia ponderosa*, 1×10^{-5} *M*; 5HT mixed responses: *Villosa lienosa*, 1×10^{-5} *M*; *Lampsilis claibornensis*, 1×10^{-6} *M*; *Corbicula manilensis*, 3×10^{-9} *M*; *Anadara tuberculosa*, 3×10^{-7} *M*. Drugs were added to the bath at the arrows. Time: 1 min.

Tables and plots

Tabulating the effects. The individual responses making up each DRP were examined for excitatory and inhibitory component effects, and the threshold for each effect was determined. Thresholds, rather than ED_{50}s, were recorded because the effects changed qualitatively with dose in many preparations. The data were compiled by species and are presented in Table I. The percentage of responding preparations affected in each of the two modes and the number of preparations failing to respond in either mode are indicated for each species. Unresponsive hearts were challenged with very high doses of agonist ($1–3 \times 10^{-5}$ *M*) and these responses (or lack of them) are also recorded in Table I.

Table I thus provides the initial characterization of the species responses to FMRFamide and 5HT. Subsequent derivations are intended to illustrate patterns among the species responses which are not immediately apparent from this characterization.

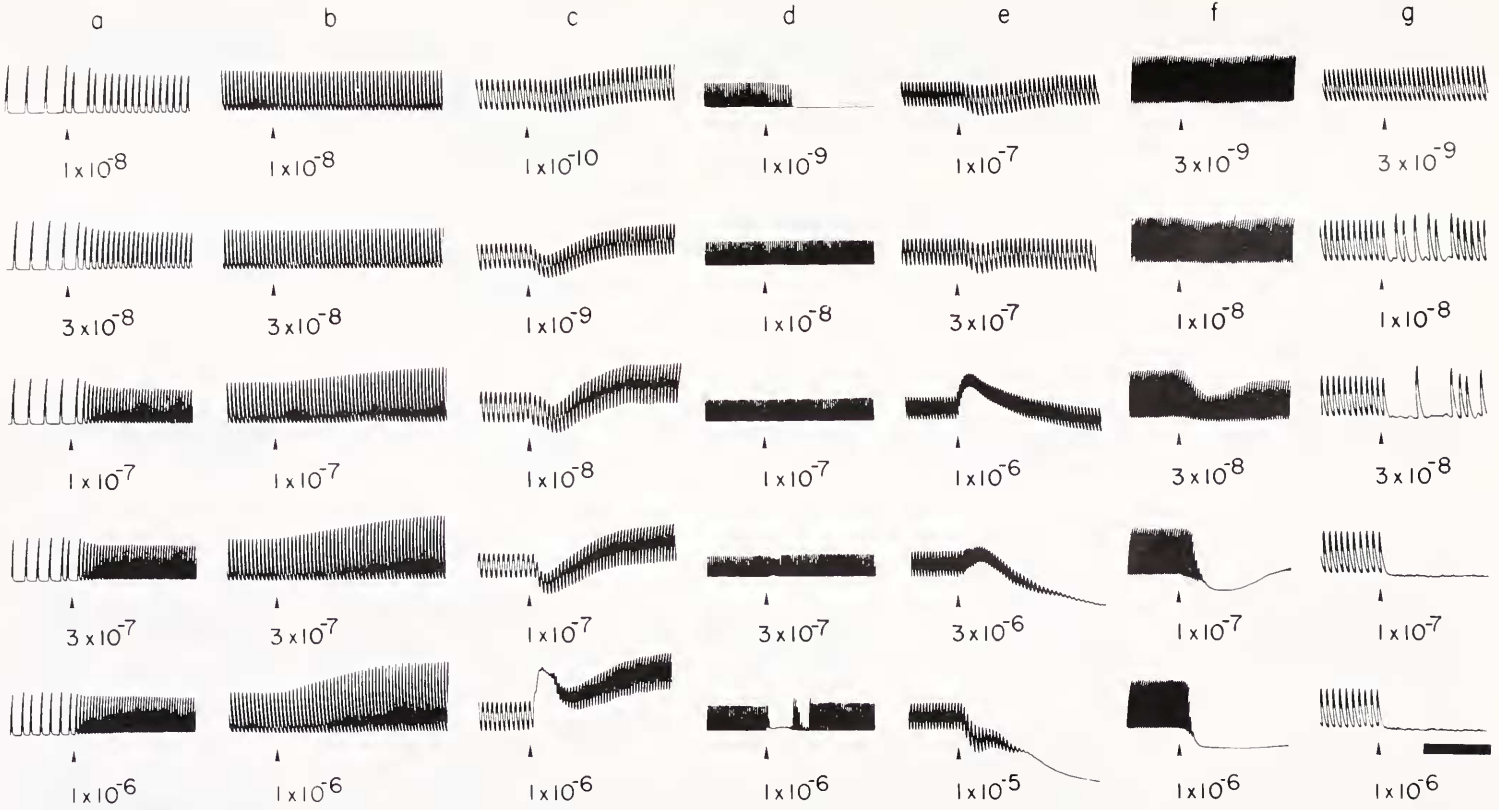


FIGURE 2. Examples of FMRFamide dose-response profiles (DRPs); each profile is from a different species. Drugs were added to the bath at the arrows; all doses are final bath concentrations. a) *Geukensia demissa granosissima*. b) *Dinocardium robustum*. c) *Corbicula manilensis*. d) *Atrina rigida*. e) *Pseudochama exogyra*. f) *Trachycardium egmontianum*. g) *Lampsilis claibornensis*. Time: 1 min.

a
b
c
d
e
1x10-9
1x10-9
1x10-9
1x10-7
7x10-9
3x10-9
3x10-9
1x10-8
3x10-7
1x10-8
1x10-8
1x10-8
1x10-7
1x10-6
3x10-8
1x10-7
3x10-8
3x10-7
3x10-6
5x10-8
1x10-6
1x10-7
1x10-6
1x10-5
7x10-8

Species response index (SRI). The SRI is a shorthand description of the species response and is derived from the distribution of DRPs among the three categories of effects outlined above (*i.e.,* excitatory, inhibitory and complex). This distribution (and therefore the SRI) is expressed in two terms. The first is the number of DRP categories observed (*i.e.,* 1–3), and this is a measure of intraspecific diversity. The second term describes the predominant effect: "e" if there were more excitatory than inhibitory DRPs, "i" if there were more inhibitory DRPs, and "c" if there were equal numbers of excitatory and inhibitory DRPs (Table II). The taxonomic distribution of SRIs by subclass is summarized in Table III.

Mean response plot. For each species and each agonist, we calculated the percentage of doses within the range of 1×10^{-10} to 1×10^{-6} *M* that produced responses with excitatory or inhibitory components. The size of the effect was not considered in these calculations. We then graphed the percentage of inhibitory effects as a function of the percentage of excitatory effects and thereby produced mean response plots (Figs. 4 and 5). Each species response is reduced to a single point in these plots. Species responses lying on the X-axes were uniformly excitatory, and those on the Y-axes uniformly inhibitory. The further the point is from the origin, the larger the percentage of effective doses and the more sensitive the species was to the agonist.

Species response curves. For each species and each agonist, we also calculated the percentages of preparations excited and inhibited by each concentration tested, from 10^{-10} to 10^{-6} *M*. The percentages were plotted against the log of the concentration, generating species response curves (Fig. 6 shows seven of them). Each species response is represented by two curves, one for excitation and one for inhibition. Of course, if the species response is uniformly excitatory (for example), then the inhibitory line will lie at Y = O.

Comparison of analytical approaches

The three analytical approaches described above provide complementary information about the species responses. For example, the SRI identifies the effect most often observed in all of the preparations of a species, and it also characterizes the variation among the DRPs. But its reliability depends strongly upon sample size. That is, we often found that the diversity of the species response would increase as we tested more preparations. The mean response plots, in contrast, display the relative contributions of the excitatory and inhibitory components to the species response, and are convenient for comparative purposes. Nevertheless, they are limited because the points are averages and concentration-independent. Finally, the species response curves display the dose-distributions of the excitatory and inhibitory effects, but they are less convenient than the mean response plots for comparing species responses.

The complementarity of the approaches becomes evident when we use all three techniques to analyze the responses of seven species with complex DRPs to both FMRFamide and 5HT (** in Table II; Figs. 6 and 7). The three methods all show that three species (*Anadara ovalis, Corbicula manilensis,* and *Macrocallista nimbosa*) have qualitatively similar responses to the two agonists, and that three others

FIGURE 3. Examples of 5HT dose-response profiles (DRPs); each profile is from a different species. Drugs were added to the bath at the arrows; all doses are final concentrations in the bath. a) *Modiolus squamosus.* b) *Corbicula manilensis.* c) *Rangia cuneata.* d) *Elliptio icterina.* e) *Geukensia demissa granosissima.* Time: a, c, d, e, 2.5 min; b, 1 min.

TABLE I

Summary of the excitatory (Exc) and inhibitory (Inh) responses of bivalve hearts to FMRFamide and 5HT.

SUBCLASS* Family Species (Number tested)	FMRFamide Response (%)	FMRFamide Threshold range (*M*)	FMRFamide U†	5HT Response (%)	5HT Threshold range (*M*)	5HT U†
PTERIOMORPHIA						
Arcidae						
Anadara lienosa floridana (2)	Exc (100)	3×10^{-9}–3×10^{-8}	0	Exc (100)	3×10^{-8}	0
Anadara tuberculosa (9)	Exc (100)	1×10^{-8}–3×10^{-7}	0	Exc (100) Inh (67)	3×10^{-9}–1×10^{-7} 3×10^{-9}–1×10^{-7}	0
Anadara ovalis (7)	Exc (100) Inh (100)	3×10^{-10}–1×10^{-7} 1×10^{-10}–1×10^{-8}	0	Exc (100) Inh (100)	1×10^{-9}–1×10^{-8} 1×10^{-10}–1×10^{-7}	0
Noetiidae						
Noetia ponderosa (7)	Exc (86) Inh (100)	1×10^{-9}–1×10^{-7} 3×10^{-9}–3×10^{-7}	0	Exc (100)	3×10^{-9}–1×10^{-7}	0
Mytilidae						
Mytilus edulis (9)	Exc (100)	3×10^{-8}–5×10^{-7}	0	Exc (100)	3×10^{-9}–3×10^{-8}	0
Mytella guyanensis (6)	Exc (100) Inh (33)	1×10^{-8}–1×10^{-7} 1×10^{-7}–3×10^{-7}	0	Exc (100)	1×10^{-9}–1×10^{-8}	0
Brachidontes recurvus (4)	Exc (100)	3×10^{-9}–3×10^{-8}	0	Exc (100) Inh (50)	1×10^{-9}–1×10^{-8} 1×10^{-7}–1×10^{-6}	0
Modiolus squamosus (7)	Exc (100)	1×10^{-9}–1×10^{-8}	0	Exc (100)	3×10^{-10}–3×10^{-9}	0
Geukensia demissa demissa (23)	Exc (100)	1×10^{-8}–3×10^{-7}	0	Exc (56) Inh (100)	3×10^{-9}–1×10^{-7} 3×10^{-9}–1×10^{-6}	0
Geukensia demissa granosissima (8)	Exc (100)	1×10^{-8}–5×10^{-8}	0	Exc (25) Inh (100)	1×10^{-8}–3×10^{-8} 3×10^{-9}–1×10^{-7}	0
Pinnidae						
Atrina rigida (10)	Exc (100) Inh (40)	3×10^{-9}–3×10^{-7} 1×10^{-9}–1×10^{-8}	0	Exc (100)	1×10^{-8}–1×10^{-6}	0
Pectinidae						
Argopecten irradians (8)	Exc (100)	3×10^{-9}–1×10^{-7}	1	Exc (100)	3×10^{-8}–1×10^{-7}	0

Limiidae						
Lima scabra (7)	Exc (100)	3×10^{-8}–3×10^{-6}	1	Exc (100)	1×10^{-8}–3×10^{-7}	0
Ostreidae						
Crassostrea virginica (31)	Exc (62) Inh (90)	3×10^{-7}–3×10^{-5} 1×10^{-6}–3×10^{-5}	10	Exc (100)	3×10^{-9}–1×10^{-6}	0
Ostrea palmula (7)	Inh (100)	3×10^{-6}–1×10^{-5}	2	Exc (100)	3×10^{-9}–3×10^{-7}	0
PALEOHETERODONTA						
Unionidae						
Lampsilis claibornensis (21)	Inh (100)	3×10^{-10}–3×10^{-8}	0	Exc (100) Inh (85)	3×10^{-10}–1×10^{-7} 3×10^{-8}–1×10^{-6}	0
Lampsilis ovata ventricosa (8)	Exc (62) Inh (88)	1×10^{-8}–1×10^{-6} 1×10^{-9}–3×10^{-7}	0	Exc (100)	1×10^{-9}–1×10^{-7}	0
Lampsilis teres (8)	Exc (100)	3×10^{-9}–3×10^{-7}	0	Exc (100)	1×10^{-9}–1×10^{-7}	0
Ligumia recta (8)	Exc (100)	3×10^{-9}–1×10^{-7}	0	Exc (100) Inh (50)	3×10^{-10}–3×10^{-8} 1×10^{-6}–1×10^{-5}	0
Ligumia subrostrata (9)	Exc (56) Inh (89)	1×10^{-7}–1×10^{-6} 1×10^{-7}–3×10^{-6}	0	Exc (100)	3×10^{-10}–3×10^{-8}	0
Villosa villosa (12)	Exc (100)	1×10^{-9}–1×10^{-8}	0	Exc (100) Inh (83)	3×10^{-10}–3×10^{-9} 3×10^{-8}–1×10^{-6}	0
Villosa lienosa (6)	Exc (100)	3×10^{-10}–1×10^{-8}	0	Exc (100) Inh (83)	3×10^{-10}–1×10^{-9} 3×10^{-8}–3×10^{-7}	0
Elliptio icterina (5)	Exc (100) Inh (100)	1×10^{-8}–1×10^{-6} 1×10^{-9}–3×10^{-8}	0	Exc (100) Inh (60)	1×10^{-9}–3×10^{-7} 3×10^{-8}–3×10^{-7}	0
Anodonta peggyae (4)	Exc (100)	3×10^{-8}–3×10^{-7}	0	Exc (100)	1×10^{-8}–3×10^{-8}	0
Anodonta cataracta (3)	Exc (100)	1×10^{-9}–3×10^{-9}	0	Exc (100)	1×10^{-9}–1×10^{-8}	0
Quincuncina infucata (5)	Exc (100) Inh (80)	1×10^{-9}–3×10^{-8} 3×10^{-7}–1×10^{-6}	0	Exc (100)	1×10^{-9}–3×10^{-8}	0
HETERODONTA						
Chamidae						
Chama pellucida (9)	Exc (100) Inh (33)	1×10^{-8}–3×10^{-7} 1×10^{-7}–1×10^{-6}	0	Exc (100)	1×10^{-9}–3×10^{-8}	0
Pseudochama exogyra (3)	Exc (33) Inh (100)	3×10^{-7} 3×10^{-9}–3×10^{-8}	0	Exc (100)	1×10^{-8}–1×10^{-7}	0

TABLE I (*Continued*)

SUBCLASS* Family Species (Number tested)	FMRFamide Response (%)	FMRFamide Threshold range (*M*)	FMRFamide U†	5HT Response (%)	5HT Threshold range (*M*)	5HT U†
Cardiidae						
Dinocardium robustum (9)	Exc (100)	1×10^{-8}–1×10^{-7}	0	Exc (100)	1×10^{-8}–3×10^{-7}	0
Trachycardium egmontianum (10)	Exc (30) Inh (100)	1×10^{-9}–1×10^{-8} 1×10^{-9}–3×10^{-7}	0	Exc (100) Inh (70)	1×10^{-9}–1×10^{-6} 3×10^{-8}–1×10^{-4}	0
Mactridae						
Rangia cuneata (21)	Exc (100) Inh (90)	1×10^{-9}–3×10^{-8} 1×10^{-10}–1×10^{-7}	0	Exc (100) Inh (100)	1×10^{-10}–1×10^{-8} 3×10^{-9}–3×10^{-5}	0
Spisula solidissima (10)	Exc (100)	3×10^{-9}–1×10^{-7}	0	Exc (100)	3×10^{-10}–3×10^{-9}	0
Tresus nuttalli (11)	Exc (100) Inh (56)	3×10^{-7}–3×10^{-6} 1×10^{-8}–1×10^{-7}	0	Exc (100)	1×10^{-9}–3×10^{-8}	0
Solenidae						
Ensis directus (5)	Exc (100) Inh (80)	1×10^{-9}–1×10^{-7} 1×10^{-9}–1×10^{-8}	0	Exc (100)	3×10^{-9}–3×10^{-8}	0
Semelidae						
Semele decisa (3)	Exc (100)	1×10^{-7}–1×10^{-6}	0	Exc (100)	3×10^{-8}	0
Semele rupicola (4)	Exc (75) Inh (75)	1×10^{-7}–3×10^{-6} 1×10^{-7}–3×10^{-7}	0	Exc (100)	1×10^{-8}–1×10^{-7}	0
Solecurtidae						
Tagelus plebeius (7)	Exc (100)	3×10^{-9}–1×10^{-7}	0	Exc (100)	1×10^{-8}–3×10^{-8}	0

Corbiculidae						
Polymesoda caroliniana (4)	Exc (100)	3×10^{-10}–3×10^{-9}	0	Exc (100)	1×10^{-9}	0
Corbicula manilensis (12)	Exc (100)	1×10^{-10}–3×10^{-9}	0	Exc (100)	3×10^{-10}–1×10^{-9}	0
	Inh (67)	1×10^{-10}–3×10^{-9}		Inh (67)	3×10^{-10}–1×10^{-9}	
Veneridae						
Mercenaria mercenaria (8)	Exc (100)	3×10^{-9}–1×10^{-7}	0	Exc (100)	1×10^{-9}–3×10^{-8}	0
Mercenaria campechiensis (11)	Exc (100)	3×10^{-10}–3×10^{-9}	0	Exc (100)	3×10^{-10}–1×10^{-8}	0
Chione cancellata (10)	Exc (100)	3×10^{-9}–5×10^{-8}	0	Exc (100)	3×10^{-9}–1×10^{-7}	0
Protothaca asperrima (7)	Exc (86)	1×10^{-9}–3×10^{-9}	0	Exc (100)	3×10^{-9}–1×10^{-7}	0
	Inh (100)	1×10^{-9}–3×10^{-8}				
Tivela stultorum (7)	Exc (100)	3×10^{-8}–3×10^{-7}	0	Exc (100)	3×10^{-9}–3×10^{-8}	0
				Inh (28)	3×10^{-7}–1×10^{-6}	
Macrocallista nimbosa (26)	Exc (100)	1×10^{-9}–3×10^{-8}	0	Exc (100)	1×10^{-9}–1×10^{-7}	0
	Inh (8)	1×10^{-7}–1×10^{-6}		Inh (29)	1×10^{-7}–1×10^{-5}	
Saxidomus nuttalli (8)	Exc (100)	1×10^{-8}–3×10^{-8}	0	Exc (100)	3×10^{-9}–3×10^{-8}	0
Dosinia discus (26)	Exc (88)	1×10^{-7}–1×10^{-5}	0	Exc (100)	3×10^{-9}–3×10^{-7}	0
	Inh (92)	3×10^{-7}–3×10^{-5}				
Dosinia elegans (2)	Exc (100)	3×10^{-5}	0	Exc (100)	3×10^{-7}–1×10^{-6}	0
	Inh (100)	1×10^{-6}–3×10^{-5}				
Myidae						
Mya arenaria (5)	Exc (100)	3×10^{-9}–3×10^{-8}	0	Exc (100)	1×10^{-9}–3×10^{-8}	0
Pholadidae						
Cyrtopleura costata (6)	Exc (100)	1×10^{-9}–3×10^{-8}	0	Exc (100)	1×10^{-9}–1×10^{-8}	0
	Inh (100)	1×10^{-9}–1×10^{-7}		Inh (67)	3×10^{-9}–3×10^{-8}	

* The taxonomy follows that outlined in Moore, 1969.

† Number of preparations failing to respond to any dose of agonist.

TABLE II

Summary of FMRFamide and 5HT dose-response profiles (DRPs) and derivation of species response indices (SRIs).*

SUBCLASS Species (Number tested)	FMRFamide DRP categories Exc	Comp	Inh	SRI	5HT DRP categories Exc	Comp	Inh	SRI
PTERIOMORPHIA								
Anadara lienosa floridana (2)	2	—	—	1e	2	—	—	1e
Anadara tuberculosa (9)	9	—	—	1e	6	3	—	2e
***Anadara ovalis* (7)	—	7	—	1c	—	7	—	1c
Noetia ponderosa (7)	—	6	1	2i	7	—	—	1e
Mytilus edulis (7)	7	—	—	1e	7	—	—	1e
Mytella guyanensis (6)	4	2	—	2e	6	—	—	1e
Brachidontes recurvus (4)	4	—	—	1e	2	2	—	2e
Modiolus squamosus (7)	7	—	—	1e	7	—	—	1e
Geukensia demissa demissa (23)	23	—	—	1e	—	14	9	2i
Geukensia demissa granosissima (8)	8	—	—	1e	—	2	6	2i
Atrina rigida (10)	6	4	—	2e	10	—	—	1e
†*Argopecten irradians* (8)	7	—	—	1e	8	—	—	1e
†*Lima scabra* (8)	7	—	—	1e	8	—	—	1e
†*Crassostrea virginica* (31)	2	11	8	3i	31	—	—	1e
†*Ostrea palmula* (7)	—	—	5	1i	7	—	—	1e
PALEOHETERODONTA								
Lampsilis claibornensis (21)	—	—	21	1i	3	18	—	2e
Lampsilis ovata ventricosa (8)	1	4	3	3i	8	—	—	1e
Lampsilis teres (8)	8	—	—	1e	8	—	—	1e
Ligumia recta (8)	8	—	—	1e	4	4	—	2e
Ligumia subrostrata (9)	1	4	4	3i	9	—	—	1e
Villosa villosa (12)	12	—	—	1e	2	10	—	2e
Villosa lienosa (6)	6	—	—	1e	1	5	—	2e

**_Elliptio icterina_ (5)	—	5	—	1c	2	3	—	2e
Anodonta peggyae (4)	4	—	—	1e	4	—	—	1e
Anodonta cataracta (3)	3	—	—	1e	3	—	—	1e
Quincuncina infucata (5)	1	4	—	2e	5	—	—	1e
HETERODONTA								
Chama pellucida (9)	6	3	—	2e	9	—	—	1e
Pseudochama exogyra (3)	—	1	2	2i	3	—	—	1e
Dinocardium robustum (9)	9	—	—	1e	9	—	—	1e
**_Trachycardium egmontianum_ (10)	—	3	7	2i	3	7	—	2e
**_Rangia cuneata_ (21)	2	19	—	2e	—	21	—	1c
Spisula solidissima (10)	10	—	—	1e	10	—	—	1e
Tresus nuttalli (11)	5	6	—	2e	11	—	—	1e
Ensis directus (5)	1	4	—	2e	5	—	—	1e
Semele decisa (3)	3	—	—	1e	3	—	—	1e
Semele rupicola (4)	1	2	1	3c	4	—	—	1e
Tagelus plebeius (7)	7	—	—	1e	7	—	—	1e
Polymesoda caroliniana (4)	4	—	—	1e	4	—	—	1e
**_Corbicula manilensis_ (12)	3	9	—	2e	3	9	—	2e
Mercenaria mercenaria (8)	8	—	—	1e	8	—	—	1e
Mercenaria campechiensis (10)	10	—	—	1e	10	—	—	1e
Chione cancellata (10)	10	—	—	1e	10	—	—	1e
Protothaca asperrima (7)	—	6	1	2i	7	—	—	1e
Tivela stultorum (7)	7	—	—	1e	5	2	—	2e
**_Macrocallista nimbosa_ (26)	24	2	—	2e	19	7	—	2e
Saxidomus nuttalli (8)	8	—	—	1e	8	—	—	1e
Dosinia discus (26)	2	21	3	3i	26	—	—	1e
Dosinia elegans (2)	—	2	—	1c	2	—	—	1e
Mya arenaria (5)	5	—	—	1e	5	—	—	1e
**_Cyrtopleura costata_ (5)	—	5	—	1c	2	3	—	2e

* DRPs can be excitatory (Exc), complex (Comp) or inhibitory (Inh).
** Species with complex DRPs to both FMRFamide and 5HT.
† Species that have one or more preparations failing to respond to FMRFamide.

TABLE III

The taxonomic distribution of species response indices (SRIs).

Agonist	Subclass	Species/SRI category							
		1e	2e	3e	1c	3c	3i	2i	1i
FMRFamide	Pteriomorphia	9	2	0	1	0	1	1	1
	Paleoheterodonta	6	1	0	1	0	2	0	1
	Heterodonta	1	6	0	2	1	1	3	0
	Totals	26	9	0	4	1	4	4	2
	(% of all species tested)	(52)	(18)	(0)	(8)	(2)	(8)	(8)	(4)
5HT	Pteriomorphia	10	2	0	1	0	0	2	0
	Paleoheterodonta	6	5	0	0	0	0	0	0
	Heterodonta	18	5	0	1	0	0	0	0
	Totals	34	12	0	2	0	0	2	0
	(% of all species tested)	(68)	(24)	(0)	(4)	(0)	(0)	(4)	(0)

(*Elliptio icterina, Trachycardium egmontianum,* and *Cyrtopleura costata*) have markedly different responses to the two agents. The complementarity of the approaches is most evident in the case of *Rangia cuneata*, however. The species responses are similarly positioned in the two mean response plots (Fig. 7), indicating that the *Rangia* hearts were excited and inhibited by about the same proportions of FMRFamide and 5HT doses. The SRIs indicate that, although inhibition was not the predominant effect, most *Rangia* preparations were inhibited by both agents (Table II). However, the species response curves differ in shape (Fig. 6); *i.e.,* 5HT inhibited only at relatively high doses, whereas FMRFamide inhibited at every dose tested. Thus, the *Rangia* hearts were similarly sensitive to 5HT inhibition but varied in their sensitivities to FMRFamide inhibition.

Pharmacological comparisons

Although both FMRFamide and 5HT were predominantly cardioexcitatory in over half of the species surveyed (SRI = 1e and 2e; Table III), neither agonist was exclusively so. Both inhibited some hearts at some doses.

The responses to FMRFamide were more variable than those to 5HT at all levels. First, a greater proportion of the FMRFamide species responses were moderately or highly diverse (SRI = 2 or 3; Table III), indicating greater variability of a single response from preparation to preparation within species. Second, there were more categories of FMRFamide SRIs and they had a broader species distribution (Table III), suggesting that the interspecific diversity of FMRFamide effects is also greater than that of 5HT effects. The points are more scattered in the FMRFamide mean response plot (Fig. 4), further supporting this conclusion.

As a corollary, the species responses to FMRFamide were less uniformly excitatory than those to 5HT. More FMRFamide species responses had inhibitory components, and inhibition was more frequently predominant (Table III). In addition, fewer points lie on the X-axis and more lie on, or close to, the Y-axis in the FMRFamide mean response plot (Fig. 4). If, in the two plots, we compare the points lying on the X-axes (N = 28 for FMRFamide and 35 for 5HT), the mean is lower for FMRFamide than for 5HT (arrowhead below each graph). Thus, on

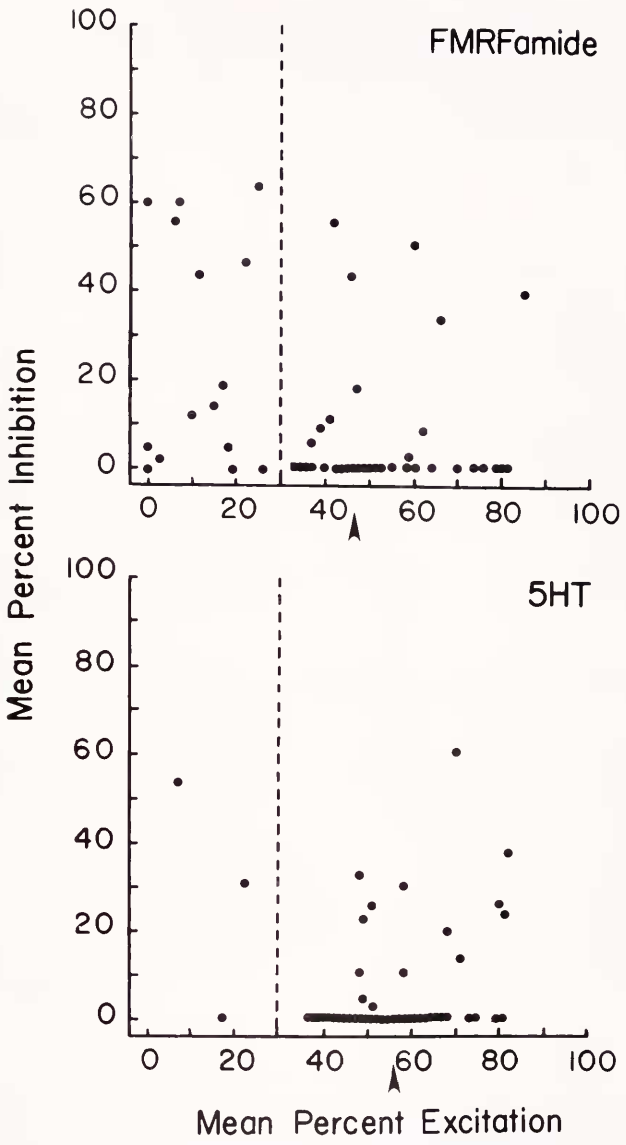
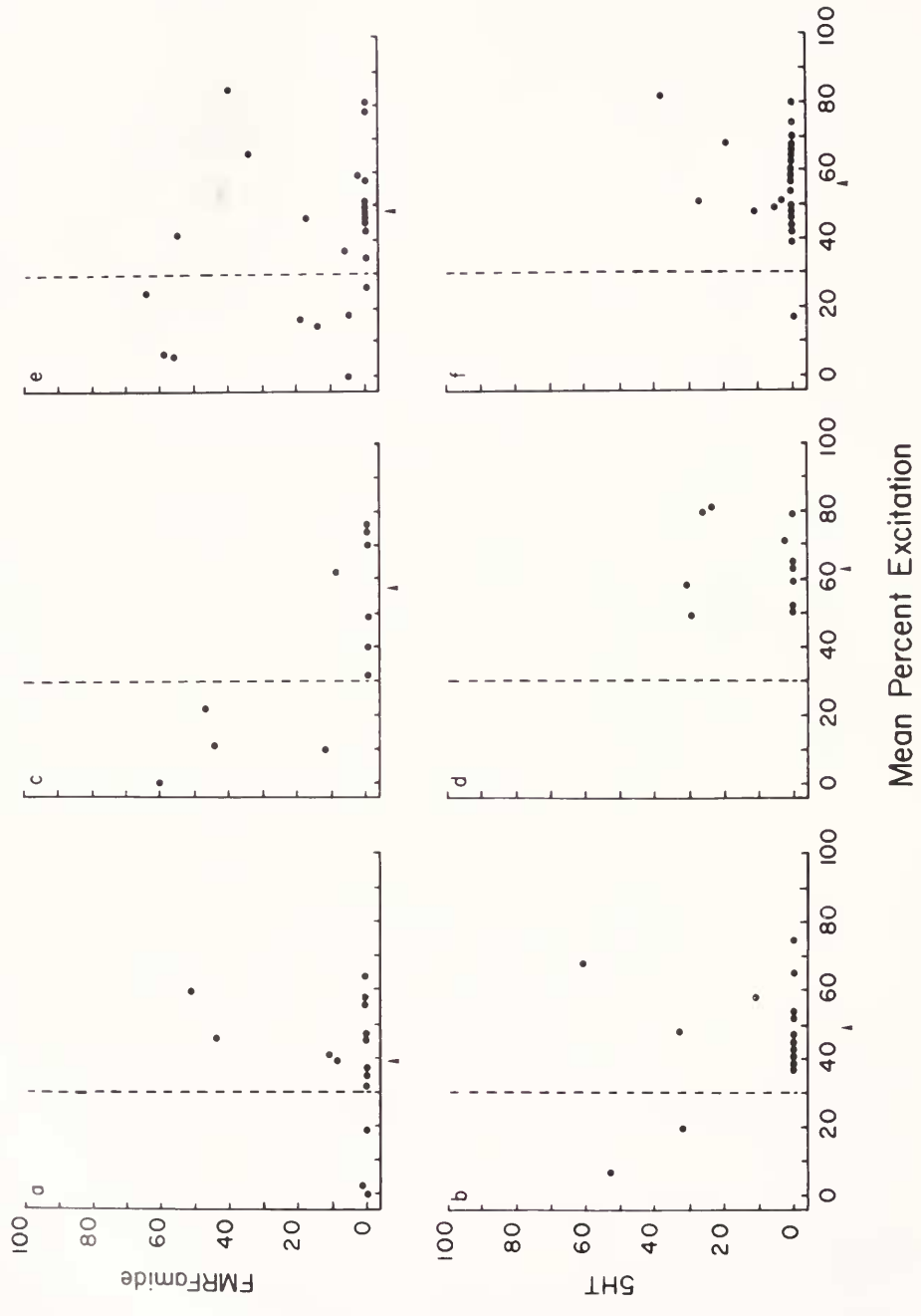


FIGURE 4. Composite mean response plots for FMRFamide and 5HT. The mean percentage of inhibitory doses is plotted as a function of the mean percentage of excitatory doses for each species. The arrowhead below each graph indicates the average mean percentage of excitatory doses for those species lying on the abscissa.

the average, fewer doses of FMRFamide were excitatory, though the difference in potency is small.

There was no consistent relationship between the responses to FMRFamide and those to 5HT. Fewer than half of the species surveyed had qualitatively similar responses to the two agonists. Of these, 20 species were solely excited by both compounds (Tables I and II); but seven of them were represented by only a few preparations. Seven species had FMRFamide and 5HT responses with both exci-


Pteriomorphia
Paleoheterodonta
Heterodonta
a
b
c
d
e
f
FMRFamide
5HT
Mean Percent Inhibition
Mean Percent Excitation
0
20
40
60
80
100

tatory and inhibitory components, but, as described above, the responses were qualitatively similar in only three of them (Table II; Figs. 6 and 7).

In conclusion, the comparisons between the responses to FMRFamide and 5HT show that neither agent is exclusively cardioexcitatory, that the effects of FMRFamide are more often inhibitory than those of 5HT, and that the variation in the responses to the two agonists is not in parallel.

Taxonomic considerations: subclasses

Phylogenetic patterns in the pharmacologies of FMRFamide and 5HT are evident at the level of subclass. However, some of the general conclusions outlined above, based on an overall comparison of the two agonists, begin to break down when the comparisons are restricted to members of a specific subclass. In particular, although the general conclusion that FMRFamide effects are less uniform and more inhibitory than those of 5HT holds for the Paleoheterodonta and Heterodonta, the relationship is exactly opposite in the Pteriomorphia. Details follow.

In both the Paleoheterodonta and Heterodonta, the FMRFamide SRIs were more disparate than those of 5HT, had a broader species distribution, and had more significant inhibitory components (Table III). Furthermore, the points were more scattered in the FMRFamide mean response plots, indicating that members of these two subclasses varied more in their average sensitivities to FMRFamide than to 5HT (Fig. 5).

In contrast, both agonists were exclusively cardioexcitatory in about two-thirds of the pteriomorphs surveyed, and both inhibited about the same proportions of species (Table III). However, the points were more scattered in the pteriomorph mean response plot for 5HT, indicating that pteriomorph hearts varied more in their average sensitivities to 5HT than to FMRFamide. Moreover, two of the three species responses lying to the left of X = 30 in the composite 5HT plot were pteriomorphs, while only three of fifteen species lying in this region of the FMRFamide graph belonged to this subclass (Figs. 4 and 5).

In summary, and as we concluded above, 5HT is rarely inhibitory or even weakly excitatory. But when such an effect does occur, it is most likely to be on a pteriomorph heart. Conversely, FMRFamide is more likely than 5HT to be inhibitory or weakly excitatory, and these effects usually occur in paleoheterodont or heterodont preparations.

Taxonomic considerations: families

Phylogenetic patterns in the pharmacologies of FMRFamide and 5HT are also discernible at the family level. The effects of FMRFamide and 5HT can be remarkably uniform within particular families, though there are usually some exceptional species. Other families have characteristically diverse species responses, especially to FMRFamide. The response characteristics of some families reflect the features of their subclass, while a few are unique. Four families illustrate this assortment of relationships; they are examined below.

Unionidae. Most extant paleoheterodonts, and all of those included in this survey, are members of the family Unionidae. The effects of FMRFamide on unionid

FIGURE 5. Mean response plots for FMRFamide and 5HT on pteriomorph (a, b), paleoheterodont (c, d) and heterodont (e, f) hearts. The mean percentage of inhibitory doses is plotted as a function of the mean percentage of excitatory doses. The arrowhead below each graph indicates the average mean percentage of excitatory doses for those species lying on the abscissa.

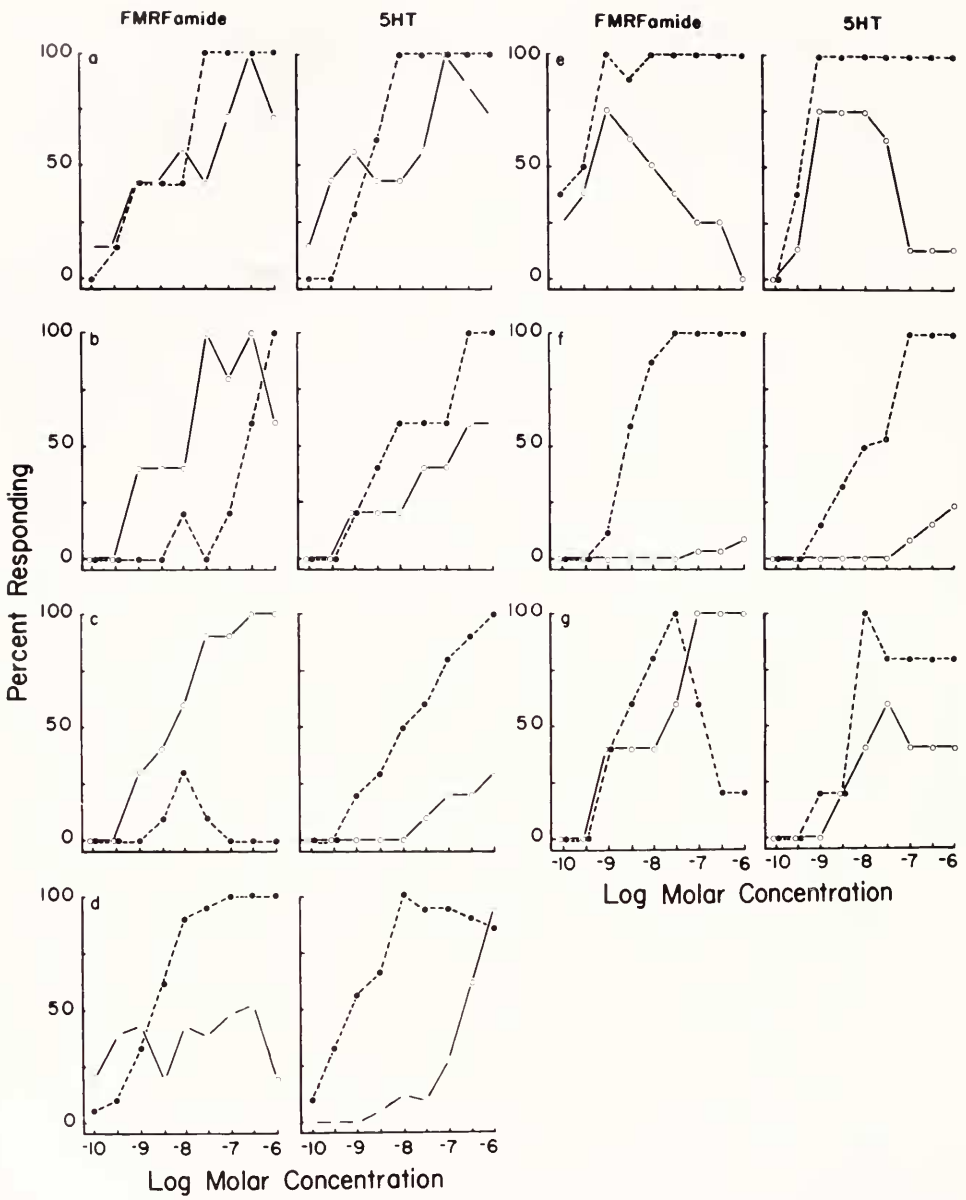


FIGURE 6. Species response curves for seven species with complex DRPs to FMRFamide and 5HT. These curves show the percentage of preparations excited (dashed line) and inhibited (solid line) by each dose of agonist between 10^{-10} *M* and 10^{-6} *M*. a) *Anadara ovalis*. b) *Elliptio icterina*. c) *Trachycardium egmontianum*. d) *Rangia cuneata*. e) *Corbicula manilensis*. f) *Macrocallista nimbosa*. g) *Cyrtopleura costata*.

hearts were characteristically diverse, even within single genera (*e.g., Lampsilis* and *Ligumia*) (Tables I, II and III). Inhibition was common, and was often a significant or predominant component of the response (*e.g., Lampsilis claibornensis*). Yet other species (*e.g., Lampsilis teres*) were only excited by the peptide.

In contrast, all unionid preparations were excited by relatively low doses of

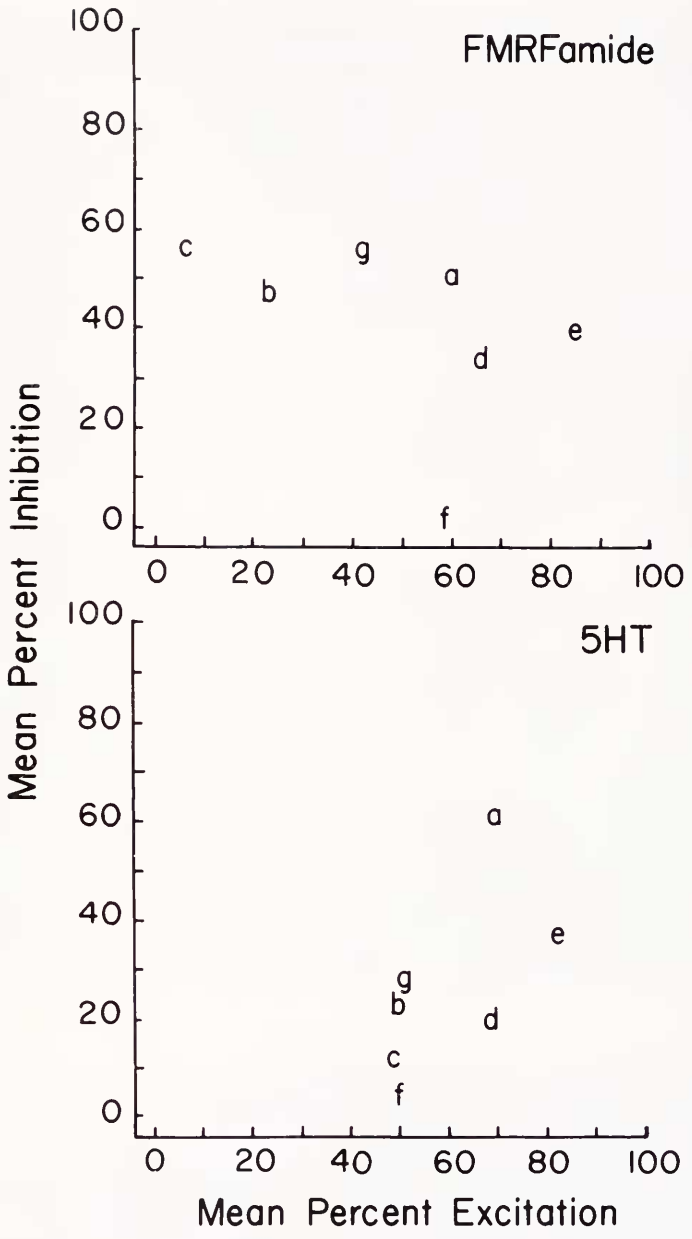


FIGURE 7. Mean response plots for seven species with complex DRPs to both FMRFamide and 5HT. The mean percentage of inhibitory doses is plotted as a function of the mean percentage of excitatory doses. Each species response is designated by a letter corresponding to that used in Figure 6. a) *Anadara ovalis.* b) *Elliptio icterina.* c) *Trachycardium egmontianum.* d) *Rangia cuneata.* e) *Corbicula manilensis.* f) *Macrocallista nimbosa.* g) *Cyrtopleura costata.*

5HT. A slowly developing inhibition appeared at higher doses in some preparations from about half of these species (Tables I, II and III). Thus, in the family Unionidae (subclass Paleoheterodonta), as in the subclass Heterodonta, the effects of 5HT are more uniform and less inhibitory than those of FMRFamide.

Veneridae. The responses of venerid hearts were not characteristic of their subclass, the Heterodonta: both FMRFamide and 5HT were overwhelmingly cardioexcitatory in this family (Tables I and II). The thresholds for excitation by both agonists were usually low (but see *Dosinia discus* and *D. elegans*; Table I). Cardioinhibition was rare and, with a single exception (FMRFamide inhibition of *Protothaca asperrima* hearts; Table I), appeared only at high doses.

Mytilidae. The singularity of pteriomorphian pharmacology is primarily a reflection of the species responses of the Mytilidae. FMRFamide was always cardioexcitatory in this family. Preparations from only one species (*Mytella guyanensis*) were ever inhibited by the peptide (Tables I and II), and the effect was transient, preceding a sustained excitation. In comparison, the effects of 5HT were more varied and often inhibitory. Inhibition appeared only at high doses in most species, but was the predominant effect in the two subspecies of *Geukensia. Geukensia* hearts were unique among all of the bivalve ventricles surveyed in this characteristic (Tables I and II). This uniqueness is also reflected in the composite 5HT mean response plot (Fig. 4): the *Geukensia* responses are the only points lying to the left of X = 30 and above the X-axis.

Ostreidae. Oyster hearts were notably insensitive to FMRFamide. None of the *Ostrea* and less than one-third of the *Crassostrea* ventricles responded to the highest FMRFamide concentrations routinely tested (1×10^{-6} *M*); and nearly one-third of the hearts from both species failed to respond to the highest doses ever tested ($1\text{–}3 \times 10^{-5}$ *M*; Table I). Only two other species (*i.e., Lima scabra* and *Argopecten irradians*), both belonging to the same order as the oysters (Pteroida), contained any preparations that failed to respond to such high doses of FMRFamide (Tables I and II). Nonetheless, compared to the oysters, these hearts were relatively responsive to the peptide (Table I). Thus, the oyster hearts are clustered alone at the origin of the FMRFamide mean response plot (Fig. 4), illustrating both the uniqueness of the effects and the efficacy of the analytical technique.

Ostreid responses to FMRFamide, when they occurred, were small and transient. *Ostrea* ventricles were only inhibited by the peptide, but *Crassostrea* ventricles were variously affected (Table II). Considering the small number of *Ostrea* hearts surveyed (N = 7, compared to 31 for *Crassostrea*), and the diversity of the *Crassostrea* species response, we suppose that further sampling of *Ostrea* preparations would also have revealed a greater diversity of effects.

Discussion

We have surveyed the effects of FMRFamide and 5HT on the mechanical activity of more than 450 ventricles from 50 species of bivalved molluscs; this is about 1% of the class Bivalvia. Considering that the objects of study were homologous organs, often from closely related species, the responses were strikingly diverse, varying qualitatively with dose as well as species. Since the usual pharmacological analyses of dose-response relationships are not well designed to deal with these kinds of variation, we developed some new approaches that allowed us to express, succinctly, the response of a preparation or species. Comparisons between drugs and taxa were made possible by the application of these techniques, and three major generalities emerged.

First, although most species responses to FMRFamide and 5HT are predominantly excitatory, both compounds inhibit the hearts of some species at some doses. Clearly, FMRFamide is not a general excitor of molluscan muscle and nerve (*e.g.,* Greenberg and Price, 1979). Ironically, the notion that it is probably arose because

the first, and most thoroughly, studied bivalve hearts were those of the atypical family Veneridae.

Second, the actions of FMRFamide and 5HT do not vary in parallel; thus FMRFamide is also not a serotonomimetic agent. Nevertheless, there are some systematic relationships between the two sets of responses which are evident at the level of subclass. In particular, FMRFamide is more likely than 5HT to be inhibitory or weakly excitatory, and these effects appear most commonly in the Paleoheterodonta and Heterodonta. In contrast, 5HT is only rarely inhibitory or even weakly excitatory; and such effects are most likely to occur in the subclass Pteriomorphia. This dichotomy between pteriomorph responses and those of the paleoheterodonts and heterodonts was not unexpected since the Paleoheterodonta and Heterodonta are more closely related to each other than either is to the Pteriomorphia (Purchon, 1978). Moreover, the pharmacological dissimilarity is in conformity with other physiological differences between pteriomorph and heterodont hearts, including the ionic bases of excitability (Deaton and Greenberg, 1980), the levels and forms of cholinesterase (Roop and Greenberg, 1976; Greenberg *et al.*, 1980), and the sodium-calcium exchange across the sarcolemma (Plumb and Koch, 1979).

Third, the responses to either FMRFamide or 5HT can be strikingly uniform in some bivalve families, and be characteristically diverse in others. Thus, although systematics and pharmacology are correlated, the effects of FMRFamide and 5HT are neither consistent nor reliable characters of bivalve families. The actions of cholinergic drugs on bivalve hearts are also loosely correlated with taxonomy (Greenberg, 1965; Greenberg *et al.*, 1980), and we suppose that, were it systematically tested, such a general correlation would be a feature of all drug-organ interactions.

Finally, several mechanisms of action undoubtedly underlie the diverse effects of FMRFamide and 5HT observed in this survey. These mechanisms remain to be investigated, however, and speculations about them, based on intensive studies of the actions of these or other drugs on the hearts of particular bivalve species (*e.g.,* Higgins *et al.*, 1978; Elliott, 1980), would probably prove to be premature. Nevertheless, this survey has provided a set of model systems which are being exploited in such investigations (*e.g.,* Painter, 1982).

Acknowledgments

This research was supported by NIH grant HL-09283 to M.J.G. It is Contribution No. 179 from the Tallahassee, Sopchoppy and Gulf Coast Marine Biological Association. We thank Drs. E. R. Stern, T. H. Dietz, and L. E. Deaton for collecting animals for us; Drs. A. Myra Keene and W. H. Heard for the identification of Panamanian and freshwater species, respectively; B. Jones and the Florida State University Department of Statistics for statistical guidance; and Drs. D. A. Price and G. T. Nagle for help collecting animals and for critical discussion of the work. We also thank Mrs. Rita Schmidt for typing the manuscript.

LITERATURE CITED

Blinks, J. R., and J. Koch-Weser. 1961. Analysis of the effects of changes in rate and rhythm upon myocardial contractility. *J. Pharmacol. Exp. Ther.* **134:** 373–389.

Deaton, L. E., and M. J. Greenberg. 1980. The ionic dependence of the cardiac action potential in bivalve molluscs: Systematic distribution. *Comp. Biochem. Physiol.* **67A:** 155–161.

Elliott, E. J. 1980. Three types of acetylcholine response in bivalve heart muscle cells. *J. Physiol.* **300:** 283–302.

GREENBERG, M. J. 1963. The effect of changes in rate on the amplitude of beat of bivalve hearts. *Proc. XVI Congr. Zool., Washington* **2:** 109.

GREENBERG, M. J. 1965. A compendium of responses of bivalve hearts to acetylcholine. *Comp. Biochem. Physiol.* **14:** 513–539.

GREENBERG, M. J., S. D. PAINTER, K. E. DOBLE, G. T. NAGLE, D. A. PRICE, AND H. K. LEHMAN. 1982. The molluscan neurosecretory peptide FMRFamide: Comparative pharmacology and relationship to the enkephalins. *Fed. Proc.* In press.

GREENBERG, M. J., AND D. A. PRICE. 1979. FMRFamide, a cardioexcitatory neuropeptide of molluscs: An agent in search of a mission. *Am. Zool.* **19:** 163–174.

GREENBERG, M. J., AND D. A. PRICE. 1980. Cardioregulatory peptides in molluscs. Pp. 107–126 in F. E. Bloom, Ed., *Peptides: Integrators of Cell and Tissue Function.* Raven Press, New York.

GREENBERG, M. J., T. ROOP, AND S. D. PAINTER. 1980. The relative contributions of the receptors and cholinesterases to the effects of acetylcholine on the hearts of bivalve molluscs. *Gen. Pharmacol.* **11:** 65–74.

HIGGINS, W. J. 1977. 5-hydroxytryptamine-induced tachyphylaxis of the molluscan heart and concomitant desensitization of adenylate cyclase. *J. Cyclic Nucleot. Res.* **3:** 293–302.

HIGGINS, W. J., D. A. PRICE, AND M. J. GREENBERG. 1978. FMRFamide increases the adenylate cyclase activity and cyclic AMP level of molluscan heart. *Eur. J. Pharmac.* **48:** 425–430.

MOORE, R. C. 1969. *Treatise on Invertebrate Paleontology*, Pt. N (Bivalvia), Vols. 1–3. The Geological Society of America, Boulder.

PAINTER, S. D. 1982. FMRFamide inhibition of a molluscan heart is accompanied by increases in cyclic AMP. *Neuropeptides* In press.

PAINTER, S. D., D. A. PRICE, AND M. J. GREENBERG. 1979. Responses of bivalve myocardia to 5-hydroxytryptamine (5HT) and the molluscan neuropeptide, FMRFamide. *Am. Zool.* **19:** 959.

PLUMB, J., AND R. A. KOCH. 1979. Sodium-dependent calcium efflux from ventricles of two bivalve molluscs. *Am. Zool.* **19:** 943.

PRICE, D. A., AND M. J. GREENBERG. 1977. Structure of a molluscan cardioexcitatory neuropeptide. *Science* **197:** 670–671.

PURCHON, R. D. 1978. An analytical approach to a classification of the Bivalvia. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **284:** 425–436.

ROOP, T., AND M. J. GREENBERG. 1976. A comparison of the cholinesterases of an oyster (*Crassostrea virginica*) and a clam (*Macrocallista nimbosa*). *J. Exp. Zool.* **198:** 121–134.

WELSH, J. H., AND R. TAUB. 1948. The action of choline and related compounds on the heart of *Venus mercenaria. Biol. Bull.* **95:** 346–353.